THE INFLUENCE OF ETHER AND ETHER ANESTHESIA ON BACTERIOLYSIS, AGGLUTINATION, AND PHAGOCYTOSIS.*

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Coincident with the rapid increase of knowledge of biological chemistry chemical explanations are being sought for many of the phenomena of immunity. In order to study the effect of a fat-solvent drug on some of these phenomena the following investigation has been carried out. Ether was used because it provided an opportunity not only for observations in test-tubes but also for a study of the conditions in the human body by virtue of its general use as an anesthetic. Moreover, the idea presented itself that the action of the ether upon the various elements of the blood and other tissues might be intimately related to the occurrence of various post-operative lung complications, a study of which was suggested to the writer by Professor Arthur Dean Bevan. The phenomena investigated have been bacteriolysis, agglutination, and phagocytosis. The clinical cases were chosen from the surgical services of the Presbyterian Hospital. Squibb's anesthetic ether has been used.

EFFECT ON BACTERIOLYSIS.

No appreciable effect of the ether on the phenomenon of bacteriolysis was revealed, as shown in the following tables. Experiments were conducted by adding ether directly to normal and immune serum and by subjecting both normal and immune serum to its action *in vivo* by inhalation. The technic employed was the usual one of counting the number of colonies on agar plates after incubating the mixtures of serum and bacteria for variable periods. The dilutions in the control tubes were made with 0.85 NaCl solution and in the others with salt solution containing ether. Sterile corks were used in the tubes instead of cotton plugs in order

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to minimize the evaporation of the ether while in the incubator; 24-hour agar cultures of the typhoid bacillus were used because of the ease with which this organism undergoes lysis. Varying amounts of ether up to 1 per cent were tested in the different experiments, but no higher percentage was tested because of the fear of destroying the bacteria by the drug itself. The following tables are representative of the results obtained:

1.—10 c.c. of normal rabbit blood were removed from the heart, defibrinated, and centrifuged; 2 c.c. of serum were put into a small test-tube and 0.05 c.c. of 10 per cent ether in 0.85 per cent NaCl solution was added to it, making the amount of ether present in the serum 0.125 per cent. In the experiment this serum is designated as "etherized" serum. To another tube 2 c.c. of serum were added to which 0.05 c.c. of 0.85 per cent NaCl solution without ether was added to be comparable with serum to which NaCl containing ether was added. This control serum is designated as "normal" serum.

TABLE 1.

	Normal Serum						Etherized Serum					
	Serum 1 c.c. NaCl. 0	Serum 0.5 c.c. NaCl 0.5 c.c.	Serum 0.25 c.c. NaCl 0.75 c.c.	Serum 0.125 c.c. NaCl 0.875 c.c.	Serum 0.0625 c.c. NaCl 0.9375 c.c.	Serum 0 NaCl 1 c.c.	Serum 1 c.c. NaCl 0	Serum 0.5 c.c. NaCl 0.5 c.c.	Serum 0.25 c.c. NaCl 0.75 c.c.	Serum 0.125 c.c. NaCl 0.875 c.c.	Serum 0.0625 c.c. NaCl 0.9375 c.c.	Serum 0 NaCl 1 c.c.
Immediate	424	640	640	648	768	1,000	500	400	480	464	520	720
After 1 hr	26	176	420	496	480	496	120	156	212	536	960	480
After 5 hrs	0	0	0	0	4	400	1	6	0	5	280	490
After 15 hrs	0	0	3	140	1,312	600	0	0	0	1,632	3,392	900

2.—5 c.c. of blood were removed from the heart of a normal rabbit which was then killed by inhalation of 5 c.c. of ether over a period of 15 minutes. Immediately after the death 5 c.c. blood were again removed from the heart. The serum obtained before death is designated as A_1; and that obtained immediately after death is designated as A_2. The experiment was undertaken immediately after collecting serum A_2.

TABLE 2.

	Rabbit A_1				Rabbit A_2			
	Serum 0.5 c.c. NaCl 0	Serum 0.25 c.c. NaCl 0.25 c.c.	Serum 0.1 c.c. NaCl 0.4 c.c.	Serum 0 NaCl 0.5 c.c.	Serum 0.5 c.c. NaCl 0	Serum 0.25 c.c. NaCl 0.25 c.c.	Serum 0.1 c.c. NaCl 0.4 c.c.	Serum 0 NaCl 0.5 c.c.
Immediately	904	1,320	1,440	880	912	1,062	760	808
After 2 hrs	34	50	112	900	68	136	152	900
After 5 hrs	3	1	14	1,000	4	0	30	1,000
After 14 hrs	0	0	0	1,200	0	0	0	1,500

3.—Human serum from a typhoid patient was used in this experiment. That portion designated as "immune serum with ether" contained 1 per cent ether in the first tube. The control serum contained 1 per cent 0.85 NaCl solution in the first tube. The sign ∞ indicates a countless number.

TABLE 3.

IMMUNE SERUM WITH ETHER.

	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	NaCl
Immediately...	128	60	20	160	80	400	640	960	800	640
After 3 hrs.....	0	0	1	4	8	560	800	1,000	480	720
After 20 hrs....	0	0	0	0	3,300	∞	∞	1,600	1,120	1,380
IMMUNE SERUM WITHOUT ETHER.										
Immediately...	204	168	52	260	396	600	664	744	704	640
After 3 hrs.....	0	0	0	0	80	320	464	800	720	800
After 20 hrs....	0	0	0	0	3,200	∞	∞	1,200	1,400	1,600

EFFECT ON AGGLUTINATION.

Here also ether seemed to have no appreciable effect. Experiments were carried out with both normal and immune typhoid serum and the typhoid bacillus. The dilutions were made with 0.85 NaCl solution. Both the macroscopic and microscopic methods were employed. Sterile corks were used instead of cotton plugs in order to prevent evaporation so far as possible. The amount of ether used in the different experiments varied from 0.1 per cent to 2 per cent in the first tube of a series. The following experiments are typical of the results obtained with the macroscopic method.

1.—Immune typhoid serum was used. Ether was added to the first tube of the series designated as "immune serum with ether," in an amount equal to 2 per cent. The first tube of the control series contained 2 per cent 0.85 NaCl solution.

TABLE 4.

IMMUNE SERUM WITHOUT ETHER.

	1:10	1:20	1:40	1:80	1:160	1:320
After 4 hrs.....	Clumped	Clumped	Clumped	Clumped	Clumped	Partially Clumped
After 20 hrs.....	"	"	"	"	"	Clumped
IMMUNE SERUM WITH ETHER.						
After 4 hrs.....	Clumped	Clumped	Clumped	Clumped	Clumped	Clumped
After 20 hrs.....	"	"	"	"	"	"

2.—This experiment was conducted similarly to that represented in the preceding table Here, however, 1 per cent ether was used instead of 2 per cent.

TABLE 5.

IMMUNE SERUM WITHOUT ETHER.

	1:10	1:20	1:40	1:80	1:160	1:320	1:640
After 4 hrs.....	Clumped	Clumped	Clumped	Clumped	Partial Clumping	Partial Clumping	No Clumping
After 24 hrs.....	"	"	"	"	Clumped	"	"

IMMUNE SERUM WITH ETHER.

After 4 hrs.....	Clumped	Clumped	Clumped	Clumped	Clumped	Partial Clumping	Partial Clumping
After 24 hrs.....	"	"	"	"	"	Clumped	"

EFFECT ON PHAGOCYTOSIS.

In this series of experiments very decided effects were obtained. The usual technic was employed in estimating phagocytosis. In general the bacterial suspensions were made from 24-hour agar cultures; and unless otherwise stated normal human leukocytes were used. The mixtures were incubated for 15 minutes at 37° C. The following table illustrates the effect *in vitro* of ether on phagocytosis.

Ether was added directly to fresh human serum in varying proportions and the opsonic power determined. The organism used was an attenuated streptococcus. The figures represent the average number of bacteria in each of 100 leukocytes counted.

TABLE 6.

Normal human serum				5.59
"	"	"	+ 1 per cent ether	3.81
"	"	"	+ 1 " " NaCl	4.92
"	"	"		2.85
"	"	"	+ 2 per cent ether	0.70
"	"	"	+ 2 " " NaCl	2.00
"	"	"		3.62
"	"	"	+ 2.5 per cent ether	1.15
"	"	"	+ 2.5 " " NaCl	2.90
"	"	"		2.53
"	"	"	+ 5 per cent ether	0.66
"	"	"	+ 5 " " NaCl	2.12

In the table it is seen that ether *in vitro* diminishes phagocytosis of streptococci, using normal serum. This diminution is less when 1 per cent ether is present than when greater amounts are used.

Because of these results a number of observations were made to determine whether or not phagocytosis as determined by the opsonic index of an individual would be affected by an ordinary

ether anesthesia. For this purpose the indices to the streptococcus of 12 individuals were taken before anesthesia and at frequent intervals afterward. The determinations were made in the usual way. The charts given below represent the opsonic index curves in several of these observations. An effort was made to choose cases which were devoid of conditions which might complicate results, such as infection, shock, hemorrhage, anemia, etc. Hence, only those cases were studied which involved comparatively simple operative procedures on apparently fairly healthy young adults. The absence of infection was determined by: (1) absence of fever,

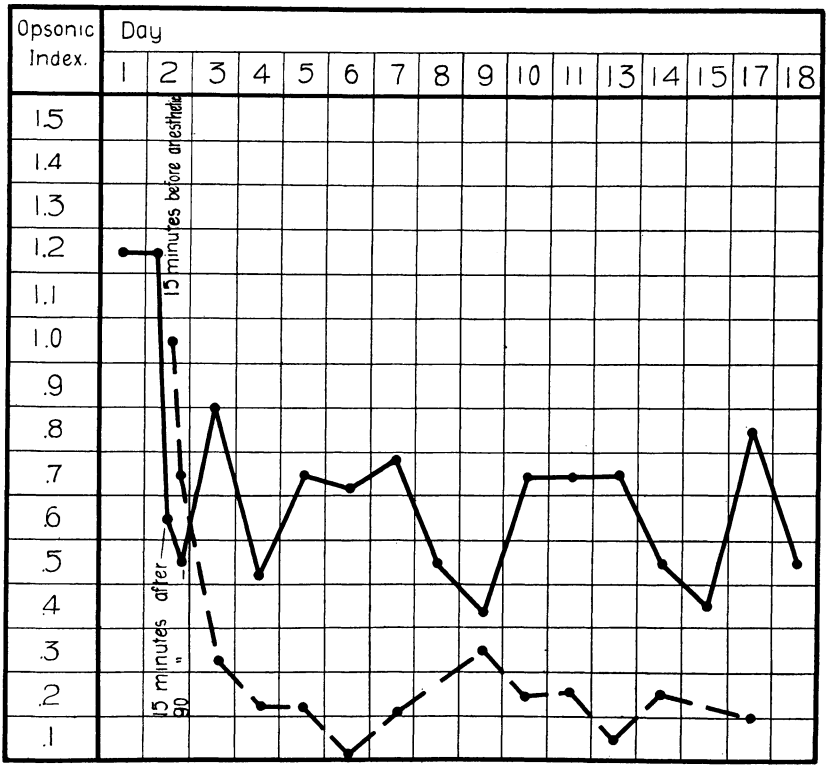

CHART 1.—Operation for bilateral inguinal hernia. Anesthesia administered for 45 minutes. Amount of ether used, 300 gm. The solid line represents the opsonic index taken in the usual way with normal leukocytes. The broken line represents the index using the patient's leukocytes instead of normal leukocytes. No observations were made after the 18th day until the 33d day. At the latter time the index was 1.1 with normal leukocytes and 0.9 with the patient's leukocytes. The index taken on "Day 1" was 24 hours before operation. On "Day 2" observations were made immediately before, 15 minutes after, and 90 minutes after respectively. This chart illustrates the longest period of depression of the index which was observed in the series.

(2) normal leukocyte counts, (3) sterility of cultures made from the wound secretion from 24 to 48 hours after operation. It may then reasonably be assumed that the results obtained are due at least in great part to the effect of the anesthesia.

In order to rule out the possibility that the results illustrated above might have been due to conditions involved in the operative procedures other than the anesthesia, three control experiments were made on normal rabbits by subjecting them solely to ether

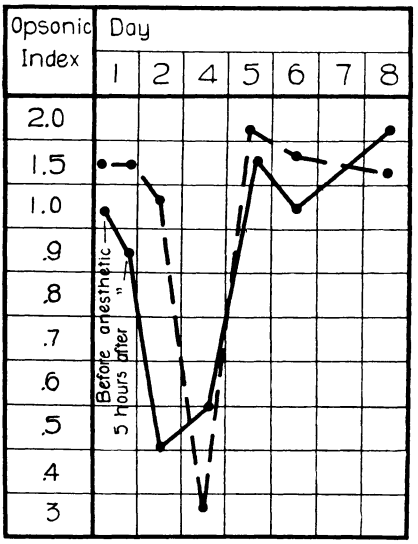

CHART 2.—Decompression operation for epilepsy. Duration, 15 minutes. Amount of ether used, 100 gm. The solid line shows the index curve with normal leukocytes; and the broken line represents the curve with the patient's leukocytes instead of normal leukocytes.

anesthesia without operation of any sort. The results obtained agree closely with those already observed in the human subject.

We see then from the above experiments that: (1) both ether *in vitro* and ordinary ether anesthesia lower markedly the phagocytic power of blood to streptococcus; (2) that this period of depression is variable, probably depending on several factors, such as the rapidity of excretion of the ether after stopping its administration, etc.; (3) that the curves of the index determinations when made with the patient's leukocytes show a greater drop than when normal leukocytes are used.

Since only the streptococcus was used it seemed to be of interest to determine whether this reduction is specific for phagocytosis of streptococci or whether the same phenomenon would hold with regard to other organisms. For this purpose observations were made in the same manner as those mentioned above, in a case of operation for inguinal hernia in a young man otherwise normal. The opsonic index of his serum to the streptococcus,

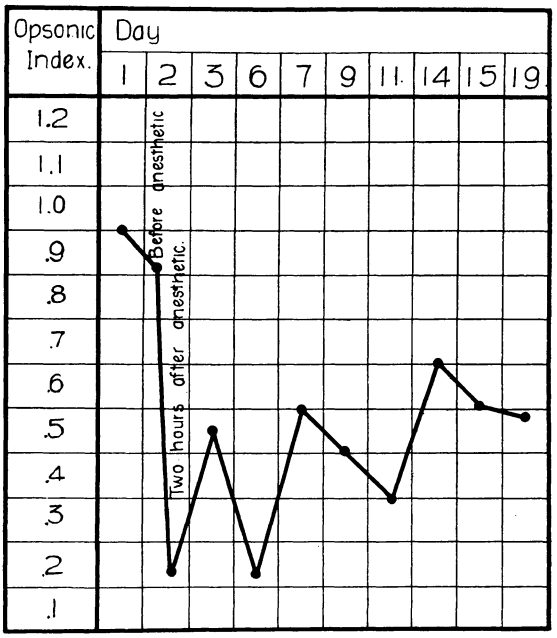

CHART 3.—Wiring of patella. Anesthetic administered for 30 minutes. Amount of ether, 200 gm. The indices were made with normal leukocytes.

pneumococcus, staphylococcus, *B. coli*, and *B. typhosus* was determined immediately before the administration of the ether and on several days afterward as shown in Chart 4. Before making the determinations in each instance both the patient's serum and the normal pool were heated in order partly to destroy complement, because of the unreliability of making opsonic determinations with the colon and typhoid bacillus when serum is used whose bacteriolytic power has not been at least partially

destroyed. Normal leukocytes were used, and the mixtures were incubated for 20 minutes. The duration of the administration of ether was 30 minutes.

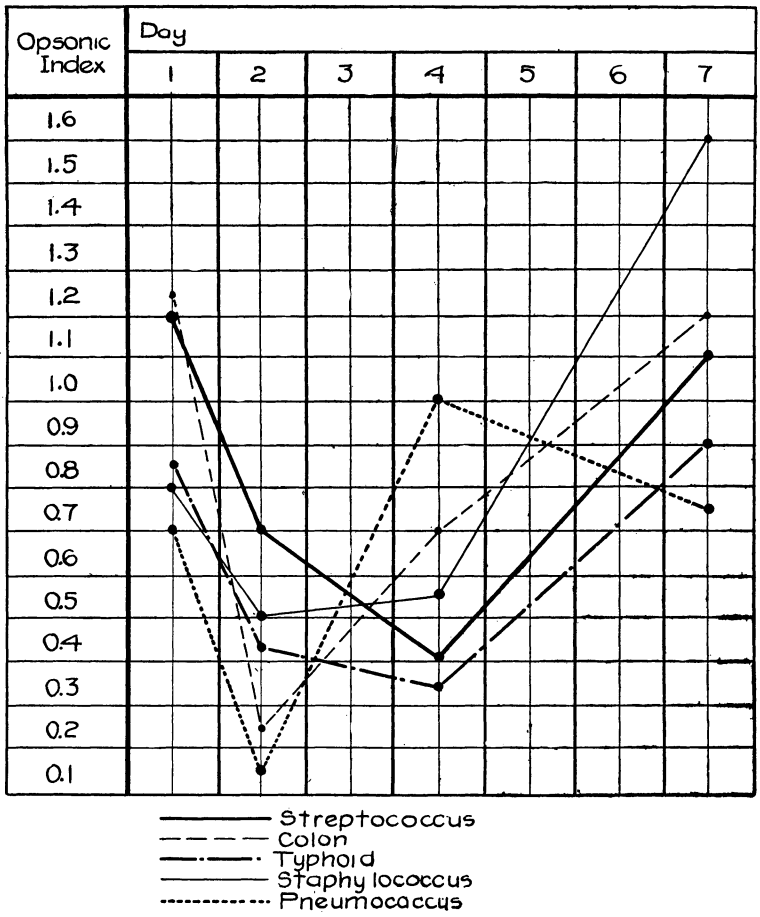

CHART 4.

Chart 5 represents a series of determinations of the opsonic index to the streptococcus and typhoid bacillus using unheated serum from a supposedly normal rabbit before and after being subjected to ether anesthesia. Normal human leukocytes were used. The solid line represents the indices to the streptococcus

and the broken line those to the typhoid bacillus. The duration of the anesthesia was 30 minutes. Why the rabbit's index to the typhoid bacillus was so high is difficult to explain. However, it may have been due either to an actual increase in typho-opsonin in the rabbit's serum or to an entirely different reason—viz., that the normal pool was more lytic for the bacillus than the animal's

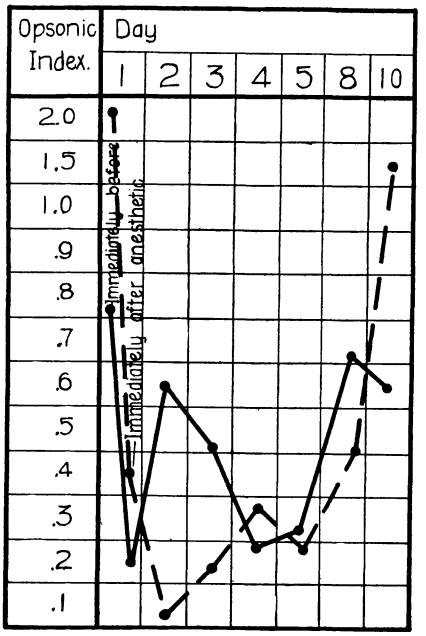

CHART 5.

serum and hence that fewer bacilli were seen in the preparations made from the normal pool.[1]

WHAT IS THE NATURE OF THE PHENOMENON?

Having found that ether reduces the phagocytic power of blood the question arose as to what part of the phagocytic system is affected, whether opsonin, leukocytes, or bacteria. The mere fact that the opsonic index is lowered does not mean necessarily that opsonin is affected, for the action may be only on the leu-

[1] The fact that a highly lytic serum may show an apparently low opsonic power has been demonstrated beautifully with reference to colon bacilli by D. J. Davis: "Immune Bodies in Urinary Infections with Colon Bacilli and Treatment by Inoculation," *Jour. Infect. Dis.*, 1909, 6, p. 224.

kocytes, the serum acting as a carrier of the anesthetic to them. The fact that when the patient's leukocytes were used there was a more pronounced reduction of the phagocytic power than when normal leukocytes were used argues for the assumption that there was some direct effect of the drug at least on the leukocytes. With this idea in view experiments were performed the object of which was to determine if possible what effect ether has upon the phagocytic activity of the white corpuscles with particular reference to their ameboid motion. Work on this point is still in progress, and the complete results will be published at a later date. However, by watching on a warm stage washed leukocytes in a suspension of carmine particles, a tentative conclusion was reached that ether inhibits the ameboid motion of the leukocytes. The results pertaining to the lowering of phagocytic power of the leukocytes *in vivo* are to be expected when it is considered that ether after being absorbed in the lungs is transported by means of the systemic circulation and that in all probability the leukocytes as well as every cell of the body are anesthetized to a more or less degree.

The next step was to determine whether or not opsonin is affected alone without reference to the leukocytes. Since bacteria are capable of being "sensitized" for phagocytosis by being placed in serum for several minutes at incubator temperature so that even if washed free from serum they remain readily phagocytable if allowed to come in contact with washed leukocytes in salt solution, it seemed as if this would afford a ready means of studying any possible effect of the ether on the serum. Accordingly the following experiment was performed: An identical amount of a suspension of streptococci was added to each of a series of tubes containing the same amount of carefully measured normal human serum. To the serum in one set of tubes was added ether in varying proportions. To the serum in the control set of tubes was added an amount of 0.85 NaCl solution equal in each case to the amount of ether which had been added to the corresponding tube. All the tubes were then incubated for one hour and later centrifuged. The supernatant fluid above the bacteria was withdrawn and the organisms were washed once in salt solution, and then resuspended in fresh salt solution. The duration of the

centrifuging process in first throwing down the bacteria and then in washing them was one hour. During this time only cotton plugs were in the tubes. Opsonic determinations were made after adding these washed streptococci to washed normal human leukocytes and incubating the mixtures for 15 minutes. The table below shows that apparently ether has in each case greatly inhibited "sensitization" and that when a large amount (25 per cent) has been used practically no sensitization has occurred. The figures represent the average number of bacteria in each of 50 leukocytes counted.

Streptococcus	sensitized	in	normal	serum	+	2	per cent	ether	2.96
"	"	"	"	"	+	2	" "	NaCl....	4.52
"	"	"	"	"	+	10	" "	ether	1.56
"	"	"	"	"	+	10	" "	NaCl....	4.38
"	"	"	"	"	+	25	" "	ether	0.20
"	"	"	"	"	+	25	" "	NaCl....	3.74

The conclusion that opsonification is inhibited by ether seems warranted in the light of these observations. But whether or not we can conclude that opsonin itself is affected directly by ether will depend on whether or not we can exclude in the interpretation of the results the following possibilities: (1) are the bacteria themselves affected by ether in such a way that they resist opsonification? or (2) is it certain that by the technic described all the ether which is at least not united with the bacteria has been removed? Concerning the first of these questions no experimental work has been undertaken in the present study, but Paul Th. Müller[1] concludes that there is no important difference in phagocytosis between bacteria extracted with ether and those not extracted. Regarding the second question, when we consider the volatility of ether and the fact that during the process of centrifugalization a considerable amount of the free ether must surely have passed off through the cotton plugs, we are led to conclude that at most only a very small amount of the drug could remain in the serum of which most, if not all, must surely have been removed by the washing, or diluted to a negligible amount by the second suspension of the organisms in fresh NaCl solution. In addition to all this is the

[1] "Einige Versuche über die Rolle der Bacterienlipoide bei der Phagocytose," *Ztschr. f. Immunitäts-forschung u. exper. Therapie*, 1908, 1, p. 61.

fact that only a small amount of the final bacterial suspension was used in making the opsonic determinations so that we can conceive that only an infinitely small proportion of the original percentage of the ether could be possibly carried over in the suspension and thus have a possible direct action upon the leukocytes. Furthermore, even if we admit that the technic was insufficient to remove all the ether, we can hardly conceive of enough remaining to cause so marked a difference as is noted when only 2 per cent of ether is used, in the light of the results seen in Table 6, where we are dealing with known quantities of the drug. We are practically forced then to the conclusion that ether has in some way affected opsonin.

Granting that opsonin is composed of both a thermolabile and a thermostable element, the next logical step was to determine if possible whether one or both of these elements were effected. Up to this time no satisfactory conclusions can be made regarding this point.

WHAT IS THE CAUSE OF THE EFFECT OF ETHER ON PHAGOCYTOSIS?

That the ether enters into no stable chemical combination with opsonin is shown by the fact that its influence may be easily done away with by bubbling a current of air through serum to which the drug has been added, by which means supposedly the ether is all removed from the serum. The following experiment, which illustrates this point, was performed: 3 c.c. of normal human serum were divided equally among three tubes. To each of two tubes ether was added in an amount sufficient to make the serum contain 2.5 per cent. Through one of these tubes containing the serum-ether mixture a current of air was passed for 40 minutes by means of a suction pump attached to a water faucet. At the expiration of that time opsonic determinations to the streptococcus were made in the usual way, using normal human leukocytes. The figures represent the average number of streptococci in each of 100 leukocytes counted:

Normal human serum	3.62
" " " +2.5 per cent ether	1.15
" " " +2.5 " " " + air	3.01

It is apparent then that the inhibitory action of the ether upon phagocytosis has been removed by means of an air stream, a fact which could hardly occur if the ether had entered into a stable combination with the serum.

In attempting to explain the cause of the effect of ether on phagocytosis the interesting hypotheses of Meyer and Overton regarding the action of the fat-solvent group of anesthetics (chloroform, ether, and alcohol) seemed to afford a possible explanation. Meyer[1] and later Overton,[2] working independently, arrived at about the same conclusions. Meyer sums up his theories in the following sentences:

1. All chemical substances, not indifferent, which are soluble in fat and fat-like bodies must be narcotic for living protoplasm in so far as they are diffusible.

2. The action will be first and strongest on those cells in whose chemical structure those fat-like substances prevail and are especially the carriers of the cell functions: in the first place then in the nerve cells.

3. The proportionate working strength of such narcotics must be dependent on their "mechanical affinity," on the one hand, for fat-like substances; on the other hand, for the remaining body constituents, particularly water; consequently on the diffusion-coefficient which determines their diffusion in a mixture of water and fat-like substances.

In view of these considerations and because of the presence of lipoids not only in the corpuscular elements of the blood but also in the serum, the idea presented itself that possibly the cause of the effect of ether on phagocytosis was in some way concerned with its solution in lipoids. Accordingly, then, experiments were performed whose object was to attempt if possible the restoration of the phagocytic power by the addition of a lipoid to serum which already contained ether. For this purpose lecithin was chosen.[3]

[1] Hans Meyer, "Zur Theorie der Alcoholnarkose, Erste Mittheilung: Welche Eigenschaft der Anaesthetica bedingt ihre narcotische Wirkung?" *Arch. f. exper. Path. u. Pharmakol.*, 1899, 42, p. 109. Fritz Baum, "Zweite Mittheilung: Ein physikalisch-chemischer Beitrag zur Theorie der Narcotica," *ibid.*, 1899, 42, p. 119.

[2] *Studien über die Narkose*, Jena, 1901.

[3] Both Merck's ovo-lecithin and a lecithin prepared from agfa and kindly furnished me by Dr. Preston Kyes were used in the experiments. No appreciable difference in the action of the two was observed.

1.—The manner of conducting the experiments was as follows: Human normal serum was divided equally among 3 tubes. To two of these tubes ether was added in a certain definite quantity. To one of these two tubes lecithin was then added and emulsified in the serum by stirring. Opsonic determinations were then made, using normal human leukocytes. The figures indicate the average number of streptococci per leukocyte.

TABLE 7.

Normal human serum	2.12
" " " + 2 per cent ether	0.74
" " " + 2 " " " + 0.2 per cent lecithin	1.52
Normal human serum	5.59
" " " + 1 per cent ether	3.81
" " " + 1 " " " + 0.2 per cent lecithin	5.25
Normal rabbit serum	3.68
" " " + 0.5 per cent ether	1.88
" " " + 0.5 " " " + 0.2 per cent lecithin	3.80

2.—In this experiment the serum used was obtained from the patient represented in Chart 2 on the second day. 50 leukocytes were counted.

Patient's serum + normal leukocytes	2.40
" " + patient's leukocytes	3.94
" " + 0.02 per cent lecithin + normal leukocytes	4.58
" " + 0.02 " " " + patient's "	6.40

3.—This experiment was conducted in a manner similar to the preceding one. Serum was taken from a patient operated upon for inguinal hernia with ether anesthesia whose chart of index curves is not given. The serum and leukocytes were collected during the period of depression of the index. 50 leukocytes were counted.

Patient's serum + normal leukocytes	1.62
" " + " " + 0.1 per cent lecithin	3.80

4.—In this experiment serum was taken from a rabbit both immediately before and immediately after anesthesia and 0.2 per cent lecithin was added to a part of it. Normal human leukocytes were used, and the determinations were made with 50 leukocytes.

Rabbit's serum before anesthesia	2.12
" " after "	0.74
" " " " + 0.2 per cent lecithin	1.52

It seems evident then in the light of these experiments that the addition of lecithin to serum containing ether is capable of at least partly restoring the phagocytic power. The question then arises, will ether saturated with lecithin cause any reduction of the phagocytosis? The following experiment is illustrative of several experiments performed to determine this point. The figures

represent the average number of streptococci in each of 150 leukocytes counted.

TABLE 8.

1 c.c. normal human serum		2.85
0.9 c.c. " " "	+ 0.1 c.c. of 20 per cent mixture of ether in NaCl (making amount ether present equal to 2 per cent)	0.70
0.9 c.c. " " "	+ 0.1 c.c. NaCl	2.00
0.9 c.c. " " "	+ 0.1 c.c. of saturated solution of lecithin in ether	2.02

The conclusion seems justified then that ether which is saturated with lecithin is practically inert and alters phagocytosis only in so far as it is a diluent of the serum.

Briefly summarized the results of these last experiments are: (1) that lecithin when added to blood containing ether is capable of restoring its phagocytic power more or less completely; and (2) that ether previously saturated with lecithin is unable to reduce phagocytosis except as it is a diluent of the serum.

These observations, however, do not warrant the conclusion that ether lowers the phagocytic power of the blood by means of a combination with the fats or lipoids of either the serum or leukocytes. For in a mixture of fluids of different solvent powers for a third substance the dissolved substance is divided between them in direct proportion to its solubility in the two fluids. Therefore, in the serum-ether-lecithin system the ether which is very much more soluble in lecithin than in water, of which the serum is in greatest part made up, will be held almost wholly in the lecithin. When the ether-lecithin mixture is put into the serum there will be practically no ether allowed to pass into the serum, and hence no effect. Nevertheless, the suggestion is strong that the effect of the ether on phagocytosis may be related to a change produced in the lipoids of the blood by the drug. For instance, it seems by no means unlikely that ether is capable of penetrating into the leukocytes and by disturbing the equilibrium of their colloid solution through a solution of their lipoids produce such changes in surface tension as will result in an alteration of activity. Moreover, since lipoids are more easily soluble in ether than in water it seems reason-

able to suppose that at least some of the lipoids of the serum may be drawn from their watery medium to the ether, and therefore that the presence of ether in the serum results in a physical change which may explain our experimental results.

Of particular interest at this point is the work of other investigators relating to the effect of other fat-solvents upon the phenomena of immunity. The well-known effect of alcoholism in predisposing to pneumonia seems perhaps to be related to this problem, particularly since by the work of Rosenow[1] and others the type of immunity in this disease appears to be largely phagocytic. Rubin[2] has shown that hypodermic injections of alcohol, ether, and chloroform in rabbits in amounts equivalent to 1½ to 2 gm. to the kilo of rabbit weight, except with chloroform the dose of which was 1 gm. per kilo, in general reduced the leukocyte counts and rendered the animals more susceptible to injections of pneumococci and streptococci, and also that *in vitro* alcohol and chloroform diminished phagocytosis of pneumococci, streptococci, and staphylococci. Deléarde[3] observed that it was difficult to immunize animals against rabies, tetanus, and anthrax after they had been made alcoholic experimentally. Laitinien[4] found that the administration of alcohol greatly hastened death in animals with experimental acute infections and rendered them much more difficult to immunize against diphtheria toxin. Abbot[5] concluded that the normal resistance of rabbits to infection with *Streptococcus pyogenes* is markedly diminished through the influence of alcohol when given daily to the stage of acute intoxication. A similar but less marked diminution of resistance to infection by the colon bacillus occurs in rabbits subjected to the same influence. Zimin[6]

[1] "Studies in Pneumonia and Pneumococcus Infections," *Jour. Infect. Dis.*, 1904, 1, p. 280.

[2] "The Influence of Alcohol, Ether, and Chloroform on Natural Immunity in Its Relation to Leukocytosis and Phagocytosis," *ibid.*, 1, p. 425.

"The Influence of Alcohol and Chloroform on Phagocytosis *in vitro*," *Jour. Am. Med. Assoc.*, 1907, 48, p. 1432.

[3] "Contribution à l'étude de l'alcoolisme expérimental et de son influence sur l'immunité," *Ann. de l'Inst. Pasteur*, 1897, 11, p. 837.

[4] "Ueber den Einfluss des Alkohols auf die Empfindlichkeit des thierischen Körpers für Infectionsstoffe," *Ztschr. f. Hyg.*, 1900, 34, p. 206.

[5] "The Influence of Acute Alcoholism on the Normal Vital Resistance of Rabbits to Infection," *Jour. Exper. Med.*, 1896, 1, p. 447.

[6] Monograph published in Russian from Tomsk Bacteriological Institute. Abstracted in *Med. Rec.*, New York, 1908, 74, p. 492.

studied the effect of ether and chloroform narcosis on phagocytosis and the bactericidal power of blood. By injecting *Proteus vulgaris* intraperitoneally into guinea-pigs he found in control animals an abundant actively phagocytic exudate with no free bacilli present in 24 to 36 hours after the injection. Of the anesthetized animals over one-half died. And in the others phagocytosis was not nearly so marked. Studies on the bactericidal properties of the blood of anesthetized and control animals gave inconstant results. Recently A. W. Kruschilin[1] by the injection into rabbits of *Staphylococcus aureus* and the spores of subtilis and anthrax bacilli has studied the effect of alcohol given intravenously in sublethal doses before the injection of the bacteria upon the destruction of these organisms by the blood *in vitro*. The technic consisted of taking cultures from the blood after inoculation at different times and comparing the number of colonies from those animals which had received alcohol with controls that had received no alcohol. In practically every case the destruction of the bacteria *in vivo* occurred much more rapidly in the control animals. Also fatal results occurred much more rapidly in alcoholized rabbits than in the controls. In animals purposely killed a short time after the injection there was always found a greater number of bacteria in the organs of the alcoholized rabbits than in the controls.

Of interest in this connection is the work of other investigators showing the probable relation of the fats and lipoids to certain other phenomena concerned in immunity. In 1902 Abbott and Bergey[2] found that the serum of rabbits which had received daily doses of alcohol for 25 days was less hemolytic for alien corpuscles than normal rabbit serum. They concluded that this action was due to a reduction of hemolytic complement in the circulating blood. Kyes[3] demonstrated that the hemolytic constituent of

[1] "Ueber die Wirkung des Alkohols auf die Tätigkeit der Phagocyten," *Ztschr. f. Immunitätsforschung u. exper. Therapie*, 1909, 1, p. 407.

[2] "The Influence of Alcoholic Intoxication upon Certain Factors Concerned in the Phenomenon of Hemolysis," *Univ. Penn. Med. Bull.*, 1902, 15, p. 186.

[3] "Ueber die Wirkungsweise des Cobragifts," *Berl. klin. Wchnschr.*, 1902, 38.
Kyes and Sachs, "Zur Kenntnis der Cobragift aktivierenden Substanzen," *ibid.*, 1903, 2.
Kyes, "Ueber die Isolierung von Schlangengiftlecithiden," *ibid.*, 1903, 42.
Kyes, "Lecithin und Schlangengifte," *Ztschr. f. physiol. chem.*, 1904, 41, p. 273.
Kyes, "Venom Hemolysis," *Jour. Infect. Dis.*, 1910, 7, p. 181.

cobra venom could be complemented very actively by lecithin. Later Morgenroth and Carpi[1] found a similar phenomenon in connection with bee poison. Neuberg[2] showed that fat-splitting substances have definite hemolytic properties, and, conversely, that the fluids containing animal, bacterial, and vegetable hemolysins contain lipolytic substances, hence presumably that the hemolysins are lipolytic. Woelfel[3] was able to extract with alcohol hemolytic substances from blood serum. Friedemann[4] and Wohlgemuth[5] demonstrated the presence in pancreatic juice of a hemolysin which could be activated by lecithin; and Noguchi[6] has found a similar hemolysin in the pancreas itself. Because he has found that soaps extracted from serum and organs act as hemolytic complements Noguchi[7] has suggested the possibility that at least some of the serum and cellular complements may be salts of the higher fatty acids with weak organic acids. Hektoen and Ruediger[8] have shown that calcium, barium, and strontium ions inactivate the complement concerned in the lysis of rabbit's corpuscles and of typhoid bacilli. As pointed out by Wells[9] these precipitate fatty acids.

Bassenge[10] has ascribed to lecithin a definite bacteriolytic property for the typhoid bacillus; but as shown by Sleeswyk[11] this property is apparently concerned with acid impurities. Vay[12] concludes

[1] "Ueber ein Toxolecithid des Bienengiftes," *Berl. klin. Wchnschr.*, 1906, 43, p. 1424.

[2] Neuberg and Rosenberg, "Lipolyse, Agglutination und Hämolyse," *ibid.*, 1907, 44, p. 54.

Neuberg and Reicher, "Lipolyse, Agglutination und Hämolyse," *Biochem. Ztschr.*, 1907, 4, p. 281; also *Munch. med. Wchnschr.*, 1907, 54, p. 1725.

[3] "Identification of Alcohol-soluble Hemolysins in Blood Serum," *Jour. Infect. Dis.*, 1905, 2, p. 97.

[4] "Ueber ein komplexes Hämolysin der Bauchspeicheldruse," *Deutsch. med. Wchnschr.*, 1907, 33, p. 585.

[5] "Untersuchungen über den Pankreassaft des Menschen," 4. Mitteilung, "Ueber ein in ihm enthaltenes komplexes Hämolysin und über die Darstellung des Lecithids," *Biochem. Ztschr.*, 1907, 4, p. 271.

[6] "Ueber eine lipolytische Form der Hämolyse," *Biochem. Ztschr.*, 1907, 6, p. 185.

[7] "Ueber gewisse chemische Komplementsubstanzen," *ibid.*, 1907, 6, p. 327.

"On Extracellular and Intracellular Venom Activators of the Blood, with Especial Reference to Lecithin and Fatty Acid and Their Compounds," *Jour. Exper. Med.*, 1907, 9, p. 436.

[8] "The Antilytic Action of Salt Solutions and Other Substances," *Jour. Infect. Dis.*, 1904, 1, p. 379.

[9] "The Present Status of Our Knowledge of the Chemistry of the Processes of Immunity," *Arch. Int. Med.*, 1908, 2, p. 262.

[10] "Ueber eine bakteriologisch interessante Eigenschaft des Lecithins," *Deutsch. med. Wchnschr.*, 1908, 34, p. 139.

[11] "Ueber die angebliche bakteriolytische Eigenschaft des Lecithins und über die Immunisierung mittels Lecithin," *Deutsch. med. Wchnschr.*, 1908, 34, p. 2263.

[12] "Ueber die immunisierende Wirkung von Lecithin—auszügen aus Pestbazillen," *Deutsch. med. Wchnschr.*, 1908, 34, p. 2265.

that lecithin in 1 and 10 per cent emulsions increases the power of agglutinating pest bacilli. Pick and Schwarz[1] found that by injections into animals of typhoid bacilli in 1 per cent lecithin emulsion a relatively high agglutinating power of the serum could be obtained in very short times. The same was true also when body lipoids (particularly leukocyte and serum lipoids) were used after being obtained by alcohol extraction.

Without going into the literature further we may conclude, then, that there is sufficient evidence already at hand to assume that the fats and lipoids play an important rôle in at least some of the immune reactions.

With the idea in view that possibly by treating serum with ether an extract could be obtained which would act as opsonic complement in reactivating heated serum, the following experiment was performed:

15 c.c. of normal rabbit serum were extracted with twice the volume of ether for 3 hours at room temperature. The ether was then removed and evaporated, after which the residue was stirred up in 1 c.c. of NaCl solution and added to heated serum. Opsonic determinations were made in the usual way, using normal human leukocytes and streptococcus. The figures represent the total phagocytosis in 50 leukocytes.

Normal serum	350
Heated normal serum	36
" " " + ether extract	30

It is evident then that ether extract of serum is unable to act as opsonic complement.

Will lecithin alone act as opsonin or as opsonic complement?

To determine the first of these points a number of experiments were performed with varying amounts of lecithin emulsified in NaCl solution. No appreciable amount of phagocytosis could be demonstrated. Table 9 (page 166) serves as a type of the results obtained.

To determine the latter point, viz., whether lecithin will act as opsonic complement, it was emulsified in varying proportions in normal human serum which had been inactivated by heat at 50° C. for 45 minutes. The experiment shown by Table 10 illustrates the negative results obtained.

[1] "Ueber die Beeinflussung der Antigenwirkung durch Lecithin und Organlipoide und deren Beteiligung am Immunisierungsprozess," *Biochem. Ztschr.*, 1909, 15, p. 453.

TABLE 9.

Normal human serum	6.40
0.85 NaCl	0.48
" " + 10 per cent lecithin	0.82
" " + 5 " " "	0.92
" " + 2 " " "	1.00
" " + 1 " " "	0.92
" " + $\frac{1}{2}$ " " "	0.95
" " + $\frac{1}{4}$ " " "	0.65
" " + $\frac{1}{6}$ " " "	0.58
" " + $\frac{1}{8}$ " " "	0.88
" " + $\frac{1}{16}$ " " "	0.75
" " + $\frac{1}{32}$ " " "	0.60
" " + $\frac{1}{64}$ " " "	0.55
" " + $\frac{1}{128}$ " " "	0.65
" " + $\frac{1}{512}$ " " "	0.90
" " + $\frac{1}{1024}$ " " "	1.00

TABLE 10.

The figures represent the average number of bacteria in each of 50 leukocytes counted.

Normal human serum	10.28
Heated normal human serum	0.36
" " " " + $\frac{1}{4}$ per cent lecithin	0.69
" " " " + $\frac{1}{2}$ " " "	0.76
" " " " + 1 " " "	0.42
" " " " + 2 " " "	0.85

Will lecithin when given in vivo after anesthesia restore the phagocytic power of the blood?

Since in the previous test-tube experiments we have seen that lecithin when added to blood whose phagocytic power has been reduced by ether will restore that power practically to a normal condition the question naturally arises as to whether the same results can be obtained *in vivo* after an anesthesia. Accordingly then a series of experiments was carried out as follows: A number of rabbits were anesthetized for varying periods of time; and immediately after stopping the anesthesia one-half of the series received an injection subcutaneously of an emulsion of lecithin in 0.85 NaCl solution. The other half of the series which served as controls received subcutaneously an equal volume of sterile NaCl solution. The lecithin was sterilized before the injections by heat for one hour at 58° C. Aseptic precautions were used when making the injections, and the site of the skin puncture in each case

was sealed with collodion. Opsonic determinations to the streptococcus were made with serum collected immediately before and immediately after the administration of the ether and then at varying periods afterward. The lecithin rabbit and its control in each instance received the same amount of anesthetic. In general rabbits of nearly the same weight were tested against each other. Where the results are expressed in terms of an index, the determinations were made by comparing the phagocytic counts of the rabbits with those of a normal human serum made with the same suspension of bacteria and leukocytes and incubated for the same length of time. Normal human leukocytes were used. The results, as will be seen in Chart 6 and the accompanying experiments, indicate that the injection of lecithin has resulted in a rather prompt restoration of the animal's phagocytic power to its preanesthetic state; whereas the controls, in general, have overcome the depression only gradually.

Two rabbits of 1,540 and 1,525 gm. weight respectively were anesthetized simultaneously with 50 c.c. of ether each over a period of 35 minutes. Immediately after stopping the inhalation of the anesthetic, serum was collected from a marginal vein of each rabbit for opsonic determinations. Rabbit A then received subcutaneously 0.1 gm. of lecithin emulsified in 2 c.c. of sterile physiological salt solution. Rabbit B at the same time was given 2 c.c. of sterile salt solution. The observations noted on the chart (page 168) represent three determinations made on the first day (before, immediately after, and four hours after the administration of the ether respectively), and one determination on each of the following three days. The solid line shows the index curve of Rabbit A, and the broken line that of Rabbit B.

Other experiments made in connection with this point are shown as follows:

1.—This experiment was conducted in a manner similar to the preceding one. Two normal rabbits of the same weight were anesthetized with 50 c.c. of ether over a period of 30 minutes. Rabbit A received 0.3 gram of lecithin in 3 c.c. of NaCl immediately after the anesthesia, and Rabbit B was injected with 3 c.c. of NaCl containing no lecithin. The figures represent the total phagocytosis.

	Before Anesthesia	Immediately after Anesthesia	24 Hours after Anesthesia	48 Hours after Anesthesia
A	350	184	178	396
B	372	140	62	130
Norman human pool	450	450	188	458

2.—Two normal rabbits of approximately the same weight, 50 c.c. of ether used with each during 40 minutes. Rabbit A received 0.5 gram of lecithin in 2 c.c. NaCl. Rabbit B received the same volume of NaCl.

	Before Anesthesia	Immediately after Anesthesia	5½ Hours after Anesthesia
Rabbit A	276	159	246
" B	258	132	132

3.—Fifty c.c. of ether administered to each for 30 minutes. Rabbit A was injected with 0.1 gram lecithin in 1 c.c. NaCl. Rabbit B received 1 c.c. NaCl.

	Before Anesthesia	Immediately after Anesthesia	4 Hours after Anesthesia	24 Hours after Anesthesia
Rabbit A	225	78	212	357
Rabbit B	248	108	187	351
Normal human pool	250	250	250	328

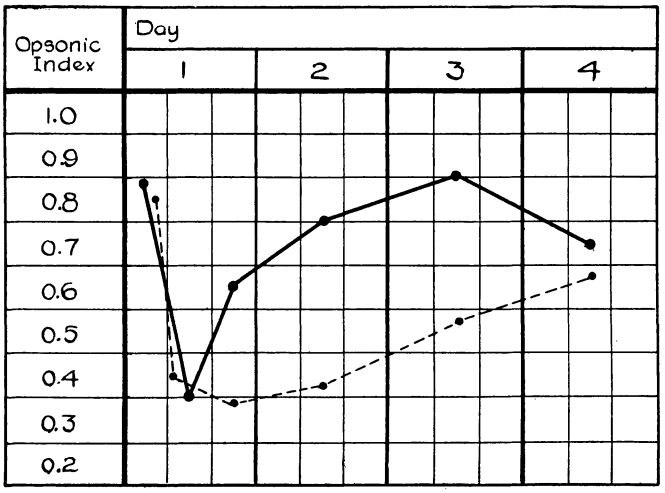

CHART 6.

In Experiments 1 and 3 the apparently marked variation in phagocytosis in all tubes which is observed after 24 hours, as compared with that obtained on the previous day with normal serum, is due merely to the fact that fresh bacterial and leukocytic suspensions were used on this day which probably varied from those of the previous day in density of both bacteria and leukocytes. On the first day when observations were made several times with each of the rabbits' sera only one determination was made with the normal human pool to compare with them; therefore the figures representing the pool are repeated to serve as a more ready means of comparison.

The explanation of how lecithin acts when given in this way is beset with many difficulties. That it is absorbed in a few hours and thereby enters the circulation to act in approximately the same manner as when in a test-tube it is stirred up in serum already containing ether, seems very unlikely in the light of the work of Henderson and Crofutt.[1] For these authors found that fats and oils were absorbed very slowly when injected subcutaneously and that usually at least several weeks were required for their disappearance. Our explanation is purely hypothetical but it seems reasonable to suppose that if when the fats of the body are nearly or completely saturated with ether, a condition which presumably exists during anesthesia, additional fat is added so that it may be within easy reach of the circulating fluids, there must be some withdrawal of the ether out of those watery media into the easily soluble fat. The effect of such a withdrawal would be then that a certain appreciable amount of ether would be removed from the serum and stored in the additional fat. This condition should consequently have two results: (1) that a certain amount of excess ether in the serum which is not held by the tissue cells because of the already existing saturation of the latter would be attracted into the fat so that temporarily at least it would be prevented from affecting any cell which had already become rid of its ether through ordinary means—as for example by becoming volatilized in the the lung and being exhaled; (2) because of the fact that ether may be stored up in the subcutaneous lecithin to be eliminated gradually, that lecithin may actually serve to hasten the elimination of the ether from the cells of both the fixed tissues and the blood. Somewhat in accord with this idea are observations reported by Nerking[2] in a preliminary communication. He has found that an intravenous injection of a suspension of lecithin shortens or even totally suppresses anesthesia. In our own work intravenous injections were not used because of the danger of not having a sufficiently fine emulsion to avoid pulmonary embolism.

The question also of how so small an amount as 0.1 gram of lecithin is capable of producing such marked changes is difficult

[1] "Observations on the Fate of Oil Injected Subcutaneously," *Am. Jour. Physiol.*, 1905, 14, p. 193.

[2] "Narkose und Lecithin (vorläufige Mitteilungen)," *München med. Wchnschr.*, 1908, 60, p. 1733.

to explain. But if, for example, we are dealing with a rabbit of 1,300 grams weight, the amount of blood contained in the animal is roughly one-thirteenth of its body weight, or 100 grams. Hoppe-Seyler's analyses of blood-plasma show that for one thousand parts of plasma there are but 1.2 parts of fat. In other words, in 100 gm. of plasma there are 0.12 gm. of fat. The 0.1 gm. of lecithin then used in the experiments would approximate the total amount of fat present in the blood of the animal and should be sufficient to exert considerable influence upon the ether present in the blood stream.

THE EFFECT OF OLIVE OIL WHEN ADDED TO ETHERIZED SERUM.

Since in the experiments dealing with lecithin we were concerned with a fat-like substance which exists almost universally in the body, it seemed of interest to determine if some fat of radically different nature would produce the same results. Accordingly the action of a purely vegetable fat was investigated and for this purpose olive oil was chosen. Experiments were conducted in a manner very similar to those with the lecithin. The results showed that, like lecithin, olive oil was capable of restoring the phagocytic power of blood in which it had been diminished by the addition of ether. One difference, however, was manifest, viz., that larger quantities of olive oil were required to produce the same results as those obtained with smaller amounts of lecithin. For the sake of brevity only one typical table is shown. The figures represent the total phagocytosis of streptococci using normal human leukocytes. The mixtures were incubated for 15 minutes:

TABLE 10.

Mixtures	Phagocytosis
Normal human serum	210
" " " + ether (2 per cent)	60
" " " + NaCl (2 per cent)	196
" " " + ether (2 per cent) + lecithin (0.1 per cent)	272
" " " + " 2 " " + olive oil (1 per cent)	202
" " " + " 2 " " + olive oil (2 per cent)	199

In view of these findings the question arose whether similar results could be obtained by giving oil *in vivo* after an ether anes-

thesia, and an effort was made to use the oil in such a manner that it could easily be employed on operative patients with no likelihood of dangerous results. For this reason the subcutaneous injection of comparatively large amounts of oil seemed impractical because of the increased danger of infection, etc. W. B. Müller[1] states that ether is excreted into the stomach after narcosis with this drug. Therefore it seemed reasonable to suppose that the introduction of the oil into the gastro-intestinal canal might produce the results in one or both of two ways: either by being at least partially absorbed and thus gaining access to the systemic circulation, or in some such way as was suggested in the attempt to explain the action of lecithin when given subcutaneously, viz., by extracting ether from the body fluids. Consequently it was decided to give the oil per rectum since this means seemed to furnish the simplest and easiest way of giving it immediately after the anesthesia. In regard to the question of how much fat can be absorbed by the large intestine there is great difference of opinion. Although Platenga,[2] Munk and Rosenstein,[2] and Deucher[2] found in their experiments that only comparatively small amounts were absorbed by the large intestine, yet, on the contrary, H. J. Hamburger[2] in a very extensive study concluded that the large intestine was able to absorb fully as much fat as the small bowel under favorable conditions. Edsall and Miller[3] in a study of two cases extending over a period of six days during which time the patients were fed entirely by rectum and received their fats chiefly as milk and eggs found that in one of these cases 33.4 per cent of the fat was absorbed and in the other 13.6 per cent.

The results of the experiments on phagocytosis using olive oil per rectum immediately after anesthesia are as follows:

Rabbits A (1,500 gm.) and B (1,475 gm.) were anesthetized, using 75 c.c. of ether for each over a period of 45 minutes. Serum was collected from each immediately before, immediately after, 3 hours after, and 24 hours after anesthesia. Rabbit A, however, received 25 c.c. of olive oil and Rabbit B 25 c.c. of NaCl solution per rectum immediately after the cessation of the administration of the ether. The figures

[1] *Narkologie*, 1, p. 338 (Berlin: R. Trenkel, 1908).

[2] Cit. by Hamburger, "Versuche über die Resorption von Fett und Seife im Dickdarm," *Arch. f. Physiol.*, 1909, p. 433.

[3] "A Study of Two Cases Nourished Exclusively per Rectum," *Univ. Penn. Med. Bull.*, Philadelphia, 1902, 15, p. 414.

in this and the following experiments represent total phagocytosis of streptococci in 100 washed normal human leukocytes.

TABLE 11.

Mixtures	Phagocytosis
Normal human pool	349
Rabbit A before ether	280
" " immediately after ether	85
" " 3 hours after ether and after receiving oil	183
Rabbit B before ether	303
" " immediately after ether	130
" " 3 hours after ether and after receiving NaCl	101
Rabbit A 24 hours after ether	133
Rabbit B " " " "	110

SIMPLE INGUINAL HERNIA OPERATION.

Duration of anesthesia was 30 minutes, at the end of which time 150 c.c. of olive oil were given per rectum.

Mixtures	Phagocytosis
Normal human pool	415
Patient's serum before ether	373
" " immediately after ether	129
" " 3 hours after ether and after oil	332

SIMPLE INGUINAL HERNIA.

Duration of anesthesia, 40 minutes; 150 c.c. of olive oil given per rectum.

Mixtures	Phagocytosis
Normal human pool	375
Patient's serum before ether	450
" " immediately after ether	187
" " 5 hours after ether and after oil	350

ENUCLEATION OF CEREBELLAR TUMOR.

Duration of anesthesia, 45 minutes; 150 c.c. of olive oil given.

Mixtures	Phagocytosis
Normal human pool	250
Patient's serum before ether	322
" " immediately after ether	137
" " 5 hours after ether and after oil	360

AMPUTATION OF LEG FOR ARTERIO-SCLEROTIC GANGRENE.

Duration of anesthesia, 20 minutes; 150 c.c. of olive oil given.

Mixtures	Phagocytosis
Normal human pool	305
Patient's serum before ether	308
" " immediately after ether	179
" " 4 hours after ether and after oil	300

TABLE 11.—*Continued.*

ENUCLEATION OF CYST OF FACE. ANESTHESIA FOR 30 MINUTES.

Mixtures	Phagocytosis
Normal human pool	248
Patient's serum before ether	273
" " immediately after ether	156
" " 2 hours after oil	235

HYSTERECTOMY FOR FIBROIDS. ANESTHESIA FOR 1 HOUR.

Mixtures	Phagocytosis
Normal human pool	210
Patient's serum before ether	184
" " immediately after ether	67
" " 3 hours after oil	168

SIMPLE INGUINAL HERNIA. ANESTHESIA FOR 25 MINUTES.

Mixtures	Phagocytosis
Normal human pool	326
Patient's serum before ether	358
" " immediately after ether	98
" " 3 hours after oil	319
" " 24 hours after oil	345
Normal human pool of day following anesthesia	355

TRANSPLANTATION OF TENDONS IN OLD BURN OF HAND. ANESTHESIA FOR 48 MINUTES.

Mixtures	Phagocytosis
Normal human pool	155
Patient's serum before ether	314
" " immediately after ether	187
" " 2 hours after oil	356

Control experiments were conducted on a series of operative patients under nearly parallel conditions except that in these 150 c.c. of physiological salt solution were given per rectum instead of oil. The following tables are typical of the results obtained:

SIMPLE INGUINAL HERNIA. ANESTHESIA FOR 30 MINUTES.

Mixtures	Phagocytosis
Normal human pool	420
Patient's serum before ether	460
" " immediately after ether	205
" " 4 hours after NaCl	121

SIMPLE INGUINAL HERNIA. ANESTHESIA FOR 35 MINUTES.

Mixtures	Phagocytosis
Normal human pool	280
Patient's serum before ether	276
" " immediately after ether	196
" " $3\frac{1}{2}$ hours after NaCl	182

TABLE 11.—*Continued.*

HYSTERECTOMY FOR FIBROIDS. ANESTHESIA FOR 1 HOUR.

Mixtures	Phagocytosis
Normal human pool	265
Patient's serum before ether	250
" " immediately after ether	198
" " 5 hours after NaCl	185

In a brief summary of these last experiments we find then that apparently the phagocytic power of blood which has been inhibited by the action of ether is at least partially restored to its preanesthetic condition within a few hours by the administration of olive oil per rectum although ordinarily as shown by Charts 1–5 the period of depression of phagocytosis extends over several days. Controls using the same amount of NaCl solution as of olive oil show no rise of phagocytosis. Whatever importance this phenomenon may have from a practical standpoint in combating the effect of ether anesthesia upon phagocytosis cannot be discussed in this article. It remains for a larger number of experiments to be performed with observation particularly, perhaps, on cases with infection.

SUMMARY AND CONCLUSIONS.

In my experiments the phenomena of bacteriolysis and agglutination were unaffected by ether.

Ether as given for anesthetic purposes reduces the property in human and rabbit blood of promoting phagocytosis of streptococcus, pneumococcus, staphylococcus, colon, and typhoid bacilli. This effect is easily studied *in vitro*.

The period of depression of phagocytosis after an ether anesthesia is variable, probably depending on many factors.

This reduction is apparently due to a direct action of the ether on the serum and the leukocytes.

There is not sufficient evidence at hand to determine which of the elements of the opsonic body of the serum is affected, whether the complement or the thermostable element.

The explanation of this action of ether is not clear, but the experiments suggest the possibility of its being due to the fat-solvent power of ether.

The ether seems not to be in any stable combination with the serum. Its inhibitory effect on phagocytosis may be removed by passing a current of air through the serum.

Lecithin in small amounts *in vitro* and also when given subcutaneously counteracts the inhibitory effects of ether. When ether is previously saturated with lecithin it has no marked inhibitory effect on phagocytosis and acts only as a diluent of the serum.

Lecithin is incapable of acting as opsonin in salt solution or of reactivating serum whose complement has been destroyed by heat.

Ether extract of serum fails to reactivate opsonin of heated serum.

Olive oil *in vitro* and *in vivo* when given per rectum seems to exert the same effect as lecithin in counteracting the inhibition of phagocytosis produced by ether, but to a less degree.

NOTE.—An abstract of this work was published in *Jour. Am. Med. Assoc.*, 1910, 54, p. 1043.